Irradiation and Modified Atmosphere Packaging for the Control of *Listeria monocytogenes* on Turkey Meat†

DONALD W. THAYER* AND GLENN BOYD

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Food Safety Research Unit, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 98-215: Received 26 August 1998/Accepted 14 May 1999

ABSTRACT

When radiation-sterilized ground turkey meat was inoculated with *Listeria monocytogenes,* packaged under mixtures of nitrogen and carbon dioxide, and irradiated with gamma-radiation doses of 0 to 3.0 kGy, there was a statistically significant ($P < 0.05$), but probably not a biologically significant, lower (0.39 log) predicted bacterial survival in the presence of 100% carbon dioxide than in the presence of 100% nitrogen. Possibly because all atmospheres contained oxygen and because a response surface design was used, gamma-radiation resistance was not significantly ($P < 0.05$) different in air than in modified atmosphere packaging (MAP) mixtures containing 5% O_2 or containing 20, 40, 60, and 80% CO_2 and balance N_2. The antilisterial effects of MAP mixtures containing 17.2, 40.5, and 64% CO_2 and balance N_2 were compared to those associated with air and vacuum packaging on turkey inoculated with approximately 5×10^3 CFU/g. Samples were irradiated to doses of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy and were stored at 7°C for up to 28 days. Irradiation treatments were significantly more lethal in the presence of air packaging than in either vacuum packaging or MAP, and in those samples that received >1.0 kGy, there was a concentration-dependent CO_2 inhibition of *L. monocytogenes* multiplication and/or recovery.

Current regulations established by the Food and Drug Administration and by the Food Safety and Inspection Service allow for the irradiation of poultry only within oxygen-permeable packaging. Yet some poultry products are currently packaged under modified atmosphere in order that a longer shelf life can be obtained. Relatively few studies have been published on the combined effects of ionizing radiation and modified atmosphere packaging (MAP) of poultry meat. Hotchkiss et al. *(15)* investigated the packaging of poultry quarters in atmospheres of 0, 60, 70, and 80% carbon dioxide (CO_2; balance air) or in permeable film-wrapped trays and found significant reductions in aerobic CFU in packages containing added CO_2 after storage for up to 35 days at 2°C. Elliott et al. *(5)* found that the combination of an atmosphere of 100% CO_2 and 2.5% added potassium sorbate was more effective than air or vacuum packaging in terms of inhibiting the growth of spoilage organisms. Marshall et al. *(19)* investigated the influence of air and MAP of 76% CO_2:13% N_2:11% O_2 or of 80% CO_2: 20% N_2 on the competition between *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked, dark-meat chicken nuggets. These authors found that at 3°C, the growth of *L. monocytogenes* was stimulated in either air or MAP containing O_2. Soffer et al. *(23)* investigated the shelf life of chicken-liver egg paté in MAP using high CO_2 and low O_2 mixtures. These authors found that the shelf life was extended from 6 days in air to 14 days in MAP. Aerobic microbial growth, mainly of *Bacillus subtilis,* was inhibited, and microbial oxidation of the product was prevented by CO_2. The presence of CO_2 is inhibitory to the growth of *L. monocytogenes* when present on various meats *(3).* Mano et al. *(18)* reported that *L. monocytogenes* grew well on turkey at 7°C in air, 100% N_2, and MAP of 20: 80% and 40:60% CO_2:O_2; however, the lag phases were extended under CO_2:O_2 MAP. Farber *(7)* reviewed the use of MAP.

Grant and Patterson *(11)* investigated the combined effects of irradiation and MAP on the survival of *L. monocytogenes* on pork and concluded that irradiated MAP pork was safer than MAP pork. The effects of irradiation and packaging of fresh meat and poultry were reviewed recently by Lee et al. *(17).* These authors concluded that more information is needed to ensure the appropriate use of vacuum or MAP in combination with irradiation of either meat or poultry. The purpose of the following study was to investigate the combined ability of gamma irradiation and MAP to control or eliminate *L. monocytogenes* on ground turkey.

MATERIALS AND METHODS

Bacterial cultures. Four isolates of *L. monocytogenes,* ATCC 7644, 15313, 43256, and 49594 (Scott A), were obtained from the American Type Culture Collection (Manassas, Va.). Cultures were maintained and isolated colonies were picked from tryptic soy agar (Difco Laboratories, Detroit, Mich.) at 37°C. Culture identity was confirmed by Gram stains and from reactions on GPI (Gram positive identification) cards using the Vitek AMS (Automicrobic System, bioMérieux Vitek, Inc., Hazelwood, Mo.) *(1).* Each isolate was cultured independently at 37°C in 100 ml of tryptic soy broth (Difco) in a baffled 500-ml Erlenmeyer culture

* Author for correspondence. Tel: 215-233-6582; Fax: 215-233-6406; E-mail: dthayer@arserrc.gov.

† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

flask agitated at 150 rpm on a rotary shaker for 18 h. Tenfold concentrations of the original inocula were prepared by mixing equal amounts of the culture of each isolate and by sedimentation of the cells by centrifugation; the concentrated cells were then resuspended in a 1 to 10 volume of sterile Butterfield's phosphate (0.25 M KH_2PO_4 adjusted to pH 7.2 with NaOH).

Radiation source, techniques, and dosimetry. The self-contained gamma-radiation source (Lockheed Georgia Company, Marrietta, Ga.) has 23 $^{137}CsCl$ pencils placed in an annular array around a 63.5-cm–high stainless-steel cylindrical chamber with a 22.9-cm internal diameter. The source strength at the time of this study was ca. 117,355 Ci (4.34 PBq) with a dose rate of 0.10 kGy min^{-1}. The dose rate was established using National Physical Laboratory (Middlesex, UK) dosimeters. Corrections for source decay were made monthly. Routine dosimetry was performed using 5-mm–diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer *(2)*. Variations in radiation dose absorption were minimized by placing small samples within a uniform area of the radiation field, by irradiating them within a polypropylene container (4-mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during each study. Samples were maintained at 5 ± 1°C during irradiation through the thermostatically controlled injection, into the top of the irradiation chamber, of the gas phase from liquid nitrogen. The polypropylene container is uniformly perforated to allow for diffusion of the cold nitrogen gas. Sample temperature was monitored continuously during irradiation with thermocouples that were taped to two samples in the chamber. Based on measurements of dosimeter responses in several experiments, the actual dose was within ±2% of the target dose.

Gas source. In the first two studies, certified commercial gas mixtures were obtained (Airco, The BOC Group, Inc., Murray Hill, N.J.; and Scott Specialty Gases, Plumsteadville, Pa.). In subsequent studies, a Smith Standard 180 SCFH Proportional Tri-Gas Blender (Watertown, S.Dak.) was used to prepare gas mixtures from certified nitrogen, carbon dioxide, and oxygen. The proper settings for the gas mixtures were determined at the start of the study via gas chromatography of each mixture.

Packaging. Two barrier pouches were used during the studies. In the earlier studies, International Kenfield (Rosemont, Ill.) All-Vak no. 13 pouches were used. This pouch film has a low oxygen permeability of 15.5 $cm^3/m^2/24$ h. In long-term studies, Mil-B-131, Type I, Class I, 5.0 mil (0.127 mm) thick polypropylene/polyethylene/aluminum foil/polyethylene barrier pouches with an O_2 transmission of 0.0093 $cm^3/m^2/24$ h (Bell Fibre Products, Columbus, Ga.) were used to provide an extremely strong barrier. In addition, in order to ensure the presence of a gas envelope over the samples, the samples were placed in the bottom of a sterile petri dish, which was placed within a 12.5 by 12.5 by 1.5-cm polystyrene meat tray. The tray containing the petri dish was placed inside the pouch. The pouches were sterilized before use by gamma irradiation (25 kGy) at ambient temperature.

A microprocessor-controlled Multivac A300 vacuum packager (Kansas City, Mo.) was used to evacuate to 40 mm Hg and to flush with an appropriate gas mixture to 700 mm Hg twice before sealing the pouch. In order to protect workers from aerosols that might contain *L. monocytogenes,* exhaust gases were passed through a HEPA filter.

Head-space gas analysis. Immediately before opening each sample pouch for purposes of microbiological analysis, a 0.5-ml sample was withdrawn from the bag with a gas-tight syringe and needle and was analyzed by gas chromatography (Gow-Mac Series 580; dual thermal conductivity detectors; ambient temperature, He carrier gas, 120 ml/min; 0.25-in. stainless-steel CTRI column [Alltech Associates, Inc., Deerfield, Ill.]). The CTR consists of an outer (6 ft by ¼ in.) column packed with activated molecular sieve and an inner (6 ft by ⅛ in.) column packed with a porous polymer mixture, designed for operation at ambient temperature using thermal conductivity detectors. The gas chromatograph was calibrated with a commercially certified gas mixture consisting of 4.58% methane, 6.99% oxygen, 15.00% carbon dioxide, and 7.04% carbon monoxide (Scotty I–no. 9799, Scott Specialty Gases, Inc., Durham, N.C.).

Substrate. Commercial ground turkey was purchased at a local grocery store and was vacuum sealed in 100-g portions in no. 400 polyethylene Stomacher bags. Each bag was then vacuum sealed within a barrier pouch. The packages of meat were rapidly frozen and sterilized with a gamma-radiation dose of 42 kGy at −30°C. The sterile meats were stored at −70°C until used.

Microbiological analysis. Initial sample dilutions were made in UVM *Listeria* enrichment broth (Difco). The enrichment broth was incubated overnight at 37°C and was then checked for the presence of viable CFU by plating on tryptic soy agar. Culture identity was confirmed as described above.

Samples were assayed for CFU by standard pour-plate procedures using tryptic soy agar, with serial dilutions in sterile Butterfield's phosphate. Each sample was diluted 10-fold and homogenized with a Stomacher Lab Blender (Model 400, Tekmar Co., Cincinnati, Ohio) for 90 s and was then pour plated in triplicate at appropriate dilutions. All samples were incubated at 37°C for 48 h. CFU were counted, at a dilution giving 30 to 300 CFU per plate, with a New Brunswick Scientific Biotran II automated colony counter.

Since the effect of CO_2 concentration in MAP on the radiation resistance of *L. monocytogenes* was unknown, a 5 by 5 central-composite, response-surface analysis *(16)* was used to determine the effects of gamma-radiation dose and atmosphere on the survival of *L. monocytogenes.* Sterile ground turkey was inoculated ($10^{9.15}$ CFU/g) and packaged in 5.0 ± 0.05 g aliquots within sterile All-Vak no. 13 pouches. Radiation doses I (0 kGy), II (0.5 kGy), III (1.0 kGy), IV (1.5 kGy), and V (2.0 kGy) and atmospheres (using certified gas mixtures) A (air), B (20% CO_2:5% O_2:75% N_2), C (40% CO_2:5% O_2:55% N_2), D (60% CO_2:5% O_2:35% N_2), and E (80% CO_2:5% O_2:15% N_2) were used. The treatment combinations were IA, IC, IE, IIB, IID, IIIA, IIIC, IIIE, IVB, IVD, VA, VC, and VE. Five samples were irradiated at the midpoint of the response-surface design IIIC (40% CO_2; 1.0 kGy). The number of surviving CFU/g was determined by counting CFU on three tryptic soy agar pour-plates, at a dilution giving 30 to 300 colonies per plate, following incubation at 37°C for 48 h. The use of sterile meat allowed us to avoid the disadvantage of a selective culture medium.

A 5 by 5 response-surface design was used to test the effects on *L. monocytogenes* of the following radiation doses: I (0 kGy), II (0.75 kGy), III (1.5 kGy), IV (2.25 kGy), and V (3.0 kGy). It was also used to test the effects on *L. monocytogenes* of the following atmospheres (using certified gas mixtures): A (100% N_2), B (27.9% CO_2:72.1% N_2), C (54.1% CO_2:45.9% N_2), D (77.9% CO_2:22.1% N_2), and E (99.0% CO_2). We used the following radiation dose–atmosphere combinations: IA, IIIA, VA, IIB, IVB, IC, IIIC, VC, IID, IVD, IE, IIIE, and VE. After inoculation, the sterile ground turkey contained $10^{9.26}$ CFU/g of the mixture of stationary-phase *L. monocytogenes.* The complete design was replicated twice.

TABLE 1. *Multiplication of* L. monocytogenes *on turkey at 7°C that was gamma-irradiated in oxygen-permeable packaging (means of two independent replicate studies, log CFU/g)*

kGy	Day (mean ± SD)								
	0	1.5	5.5	8.5	12.5	14.5	20.5	22.5	27.5
0	3.73 ± 0.00	4.12 ± 0.29	6.08 ± 0.25	7.65 ± 0.04	8.78 ± 0.46	8.96 ± 0.39	9.11 ± 0.06	9.10 ± 0.25	9.25 ± 0.20
0.5	1.26 ± 0.06	1.28 ± 0.21	3.04 ± 0.80	4.63 ± 0.42	6.28 ± 1.47	6.00 ± 0.12	7.56 ± 0.29	6.53 ± 1.02	7.34 ± 0.10
1.0	0.82 ± 0.00	0.00 ± 0.00	0.67 ± 0.21	1.69 ± 1.22	5.87 ± 0.56	3.71 ± 0.14	4.32 ± 0.80	4.29 ± 0.09	6.05 ± 0.05
1.5	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.67	0.00 ± 0.00	0.00 ± 0.00	0.97 ± 0.64	0.41 ± 0.58	0.00 ± 0.00	2.18 ± 3.09
2.0	0.00 ± 0.00	0.26 ± 0.37	0.52 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.41 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

We determined the effects of the following on the survival and growth of *L. monocytogenes* (on 25-g samples of ground turkey, samples that were incubated at 7°C for up to 28 days): gamma-radiation doses of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy and packaging atmospheres of air, vacuum, and 17.2, 40.5, or 64.0% CO_2 (balance nitrogen). One hundred grams of sterile ground turkey was inoculated with sufficient inoculum for an average of $10^{3.74}$ CFU/g (after being mixed with the meat). In replicate 1, samples were withdrawn at 0, 2, 6, 9, 13, 15, 21, 23, and 28 days. In replicate 2, samples were withdrawn at 0, 1, 5, 8, 12, 14, 20, 22, and 27 days, as dictated by scheduling needs. For analysis, these values were averaged to 0, 1.5, 5.5, 8.5, 12.5, 14.5, 20.5, 22.5, and 27.5 days; we expected small differences between results at 7°C at 1-day intervals. The actual CO_2 concentrations were 17.2 ± 3.3, 40.5 ± 4.5, and 64.0 ± 6.0%. Enrichment cultures were performed in order to verify the presence or absence of viable *L. monocytogenes*. The initial population level of *L. monocytogenes* in the ground turkey following inoculation was $10^{3.73}$ CFU/g. The complete design was replicated twice.

Statistical analyses. The means of triplicate plate counts were converted to log CFU/g. In order to facilitate log analysis, CFU values of 0 were assigned a value of 1. The means and population-reduction data were analyzed, and regressions were tested for differences by analysis of variance, using the general linear model (GLM) procedure of the SAS statistical package *(10, 22)*. Regression techniques were used to fit second-order response-surface models to the data in order to predict the number of survivors following a given treatment *(4)*.

RESULTS

The initial response-surface study with air or MAP of 20, 40, 60, and 80% CO_2:5% O_2:balance nitrogen did not reveal significant ($P < 0.05$) differences in the sensitivity of *L. monocytogenes* to gamma radiation due to CO_2 or to the presence of air. The log CFU values for the analysis were as follows: for atmosphere (atm) air: 0 kGy, 9.15; 1 kGy, 8.06; 2 kGy, 6.27; for atm 20% CO_2: 0.5 kGy, 9.00; 1.5 kGy, 7.35; for atm 40% CO_2: 0 kGy, 9.38; 1 kGy, 8.18 ± 0.42; 2 kGy, 6.58; for atm 60% CO_2: 0.5 kGy, 8.80; 1.5 kGy, 7.42; and for atm 80% CO_2: 0 kGy, 9.46; 1 kGy, 7.93. Air was included as one of the atms in order to provide a reference to the results that would be expected of standard poultry pouches and of all MAP mixtures containing 5% O_2. Though these results tended to indicate that the lethality of gamma radiation for *L. monocytogenes* was not greater in the presence of CO_2, we concluded that the presence of oxygen might have influenced these results and designed the next study using mixtures of N_2 and CO_2. In this study, we relied on the supplier's certification for the gas mixtures and did not analyze gas mixtures in the packages, a step that was taken in later studies. Thus, it is probable that the gas mixtures in the packages differed from the supply gas. It is also possible that the use of a response-surface design as opposed to the actual determination of radiation *D* values (radiation dose required to inactivate 90% of the cells) may have decreased our ability to detect small differences in radiation resistance. However, the purpose of a response-surface design is to identify large differences.

Significant ($P < 0.05$) effects were noted for atmosphere, kGy, atmosphere × kGy, and atmosphere^2 in terms of the survival of *L. monocytogenes* in MAP containing 0 to 100% CO_2 in two studies. The log CFU values for the analysis were as follows: for atm 100% N_2: 0 kGy, 9.26 ± 0.02; 1.5 kGy, 7.83 ± 0.00; 3 kGy, 5.06 ± 0.15; for atm 27.9% CO_2: 0.75 kGy, 8.60 ± 0.07; 2.25 kGy, 6.28 ± 0.09; for atm 54.1% CO_2: 0 kGy, 9.22 ± 0.04; 1.5 kGy, 7.72 ± 0.03; 3.0 kGy, 4.64; for atm 77.9% CO_2 (only one sample available): 0.75 kGy, 8.56 ± 0.05; 2.25 kGy, 6.19 ± 0.02; and for atm 99%: 0 kGy, 9.22 ± 0.12; 1.5 kGy, 7.73 ± 0.01; 3.0 kGy, 4.64 ± 0.00. The computations used the measured rather than the target amounts of CO_2. The actual amounts of CO_2 were 0, 27.9 ± 1.5, 54.1 ± 1.9, 77.9 ± 1.8, and 99.0 ± 1.4%. The term atmosphere^2 was not significant in the first analysis of variance calculation and was eliminated from the model statement. The following response-surface equation, with an R^2 value of 0.996, was generated: log CFU = 9.2321 + 0.0001 × atmosphere − 0.5324 × kGy − 0.0013 × atmosphere × kGy − 0.2973 × kGy^2. The permitted values for atmosphere were 0 to 100% CO_2 and for kGy were 0 to 3.0 kGy. There were minor differences in survival because of increased CO_2 in the packaging atmosphere; for example, at 0 and 100% CO_2, the equation predicts survivors of 4.57 and 4.96 CFU/g, respectively, following a radiation dose of 3.0 kGy. This is a predicted difference of only 0.39 CFU/g. The results indicated that high CO_2 concentrations in the MAP would produce slightly greater lethality of *L. monocytogenes* as a result of gamma radiation. The very small multiplier (0.0001) for the atmosphere term indicates that this effect is small, but the analysis of variance indicates that it is significant ($P < 0.05$) for the atmosphere term and also for the atmosphere × kGy terms ($P < 0.02$). Differences that

TABLE 2. *Multiplication of* L. monocytogenes *on ground turkey at 7°C that was vacuum-packaged and gamma-irradiated (means of two independent replicate studies, log CFU/g)*

	Day (mean ± SD)								
kGy	0	1.5	5.5	8.5	12.5	14.5	20.5	22.5	27.5
0	3.81 ± 0.02	4.08 ± 0.13	5.51 ± 0.41	7.46 ± 0.06	8.24 ± 0.12	8.42 ± 0.40	8.40 ± 0.04	8.60 ± 0.29	8.71 ± 0.12
0.5	2.44 ± 0.76	1.42 ± 2.01	4.43 ± 0.66	5.15 ± 0.27	6.27 ± 1.10	6.79 ± 1.76	8.31 ± 0.08	8.24 ± 0.24	8.65 ± 0.29
1.0	2.37 ± 0.25	1.78 ± 0.37	2.94 ± 1.64	5.08 ± 0.17	5.89 ± 0.45	5.63 ± 1.59	6.94 ± 1.42	7.68 ± 0.91	8.40 ± 0.04
1.5	0.82 ± 1.16	0.86 ± 1.22	1.38 ± 1.21	3.43 ± 0.78	4.69 ± 0.25	4.39 ± 1.48	5.59 ± 0.38	5.12 ± 0.00	8.03 ± 0.59
2.0	1.17 ± 0.07	0.00 ± 0.00	0.61 ± 0.86	1.28 ± 1.81	3.71 ± 0.02	2.20 ± 2.37	2.74 ± 0.58	0.00 ± 0.00	2.82 ± 4.00
2.5	0.67 ± 0.21	0.00 ± 0.00	0.26 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	3.93 ± 1.48	0.00 ± 0.00	0.00 ± 0.00	2.85 ± 4.02

are less than 1 log are generally considered not to be significant in biological systems.

Analysis of variance of the results of two studies obtained during 27.5 days of storage of ground turkey (at 7°C) that was inoculated with *L. monocytogenes* and that was packaged in air, vacuum, and MAP mixtures consisting of 17.2 ± 3.26, 40.5 ± 4.5, and 64.0 ± 6.1% CO_2 produced the following significant results. The probabilities of greater values of F, with an R^2 value of 0.894, were noted to correspond to the effects of the following: atmosphere (0.0001), kGy (0.0001), day (0.0001), kGy × atmosphere (0.0001), day × atmosphere (0.0001), kGy × day (0.0001), kGy × day × atmosphere (0.0024), day^2 (0.0298), kGy^2 × atmosphere (0.0001), and day^2 × atmosphere (0.0002). Analysis of covariance indicated that each atmosphere produced significantly ($P < 0.0001$) different results. The results and the analysis of variances for each individual atmosphere are reported in Tables 1 through 6. Concentrations of 40.5 and 64.0% CO_2 inhibited the multiplication and extended the lag phase of nonirradiated *L. monocytogenes* (Tables 4 and 5) compared to air-, vacuum-, or 17.2% CO_2-packed samples (Tables 1 through 3). In an atmosphere containing 64.0% CO_2, the lag phase was extended from approximately 5 days at 0 kGy to 20 days at a dose of 1.0 kGy (Table 4). These results are also indicated by the progressively lower values for the day and kGy × day terms as the CO_2 concentration increases from 17.2 to 64%. More radiation lethality occurred in air than in MAP or in vacuum (Tables 1 through 5); this is indicated by the significantly greater values for the kGy and kGy^2 terms for oxygen-permeable packaging and by the decreased survival of *L. monocytogenes* in the irradiated samples at day 0 compared with those for any of the other atmospheres. At the start of the study, enrichment cultures detected surviving *L. monocytogenes* in all samples except those that received a dose of 2.0 or 2.5 kGy in oxygen-permeable packaging. At the end of the study, with both replicates, some of the samples that had received doses as high as 2.5 kGy remained positive for viable *L. monocytogenes* by enrichment culture. The obvious exceptions were those samples that were irradiated in oxygen-permeable packaging to an absorbed dose of 2.5 kGy, of which only a few samples contained viable *L. monocytogenes.*

DISCUSSION

There is a well-established body of literature *(6, 14)* that indicates that most microorganisms are more sensitive to the effects of ionizing radiation in the presence of oxygen; however, Hastings et al. *(13)* discovered that isolates of *Lactobacillus sake, Lactobacillus curvatus,* and *Lactobacillus alimentarius* had lower gamma-radiation D_{10} values on minced meat when it was packaged under CO_2 than when it was packaged under N_2. Zelle and Hollaender *(26)* concluded that the gas (CO_2, N_2, He, and H_2) used to replace oxygen was of no importance. Our response-surface study indicated that *L. monocytogenes* on turkey meat was neither more nor less sensitive to radiation under MAP of 20, 40, 60 or 80% CO_2 in the presence of 5% O_2, nor was it more sensitive within oxygen-permeable packaging. A low level of oxygen was included in this study because various investigators have concluded that it is difficult, if not impossible, to remove the last traces of oxygen from MAP meats.

In the next study we compared the relative effects of N_2 and CO_2 atmospheres on the lethality of gamma radiation for *L. monocytogenes.* Analysis of head-space gas in

TABLE 3. *Multiplication of* L. monocytogenes *on turkey at 7°C that was packaged and gamma-irradiated in an atmosphere of 17.2% CO_2 and 82.8% N_2 (means of two independent replicate studies, log CFU/g)*

	Day (mean ± SD)								
kGy	0	1.5	5.5	8.5	12.5	14.5	20.5	22.5	27.5
0	3.73 ± 0.05	3.81 ± 0.09	5.33 ± 0.17	6.74 ± 0.21	7.63 ± 0.30	8.14 ± 0.78	8.34 ± 0.05	8.61 ± 0.07	8.69 ± 0.06
0.5	2.94 ± 0.06	3.10 ± 0.01	4.16 ± 0.30	5.26 ± 0.36	6.38 ± 1.29	6.83 ± 1.10	8.30 ± 0.12	8.52 ± 0.17	8.54 ± 0.11
1.0	2.35 ± 0.64	2.53 ± 0.25	3.02 ± 1.27	4.20 ± 0.20	6.21 ± 0.09	5.46 ± 1.54	6.40 ± 1.12	7.53 ± 0.28	8.53 ± 0.12
1.5	2.08 ± 0.02	1.15 ± 0.21	1.61 ± 0.44	3.40 ± 0.37	4.96 ± 0.33	4.44 ± 1.09	5.36 ± 0.68	6.07 ± 0.00	7.99 ± 0.00
2.0	0.50 ± 0.71	0.41 ± 0.58	0.41 ± 0.58	0.67 ± 0.21	3.17 ± 0.15	3.46 ± 0.68	3.38 ± 0.36	3.48 ± 0.00	5.16 ± 0.68
2.5	0.26 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.86 ± 1.22	0.93 ± 1.32	0.26 ± 0.37	0.80 ± 1.13	2.67 ± 0.00	2.60 ± 3.68

TABLE 4. *Multiplication of* L. monocytogenes *on turkey at 7°C that was packaged and gamma-irradiated in an atmosphere of 40.5% CO_2 and 59.5% N_2 (means of two independent replicate studies, log CFU/g)*

	Day (mean ± SD)								
kGy	0	1.5	5.5	8.5	12.5	14.5	20.5	22.5	27.5
0	3.77 ± 0.04	3.90 ± 0.12	4.64 ± 0.59	5.87 ± 0.01	6.97 ± 0.11	7.43 ± 1.50	8.03 ± 0.09	8.40 ± 0.23	8.56 ± 0.14
0.5	2.89 ± 0.23	3.00 ± 0.09	3.29 ± 0.04	4.85 ± 0.93	5.79 ± 1.13	6.33 ± 1.41	7.69 ± 0.56	8.16 ± 0.22	8.36 ± 0.12
1.0	2.64 ± 0.34	2.58 ± 0.22	2.69 ± 0.36	3.87 ± 0.82	4.89 ± 0.63	4.51 ± 1.21	5.92 ± 0.18	7.25 ± 0.36	8.13 ± 0.42
1.5	2.07 ± 0.06	1.40 ± 0.38	1.18 ± 0.26	2.18 ± 0.07	4.04 ± 0.74	3.55 ± 0.21	3.79 ± 1.12	4.85 ± 0.00	6.79 ± 0.79
2.0	0.26 ± 0.37	0.26 ± 0.37	0.26 ± 0.37	0.82 ± 0.42	2.67 ± 1.26	1.86 ± 1.04	2.50 ± 1.94	2.48 ± 0.00	4.91 ± 0.06
2.5	0.26 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.91 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

the packages did not reveal the presence of oxygen in 22 of the 26 samples. In the four samples containing measurable oxygen, those concentrations ranged from 0.06 to 0.71%. We expected, on the basis of our first study, to find no difference in radiation sensitivity with either gas. Instead, we discovered a small but significant increase in the sensitivity of *L. monocytogenes* on turkey under an atmosphere of 100% CO_2. Patterson *(20)* found increased sensitivities of *Escherichia coli* and *Salmonella typhimurium* to ionizing radiation in vacuum or CO_2 compared with those in air. Those results seem, however, to vary from those of others who have noted increased radiation lethality for *Salmonella* in the presence of oxygen *(24)*. Our first two studies used response-surface modeling techniques to estimate the immediate effects of CO_2 on the lethality of ionizing radiation for *L. monocytogenes* when it was irradiated on ground turkey meat. The authors do not consider the results of the first two studies to be biologically different. Neither of these studies, however, provided insight into the possible interaction of CO_2 with the recovery of radiation-injured cells during storage at refrigeration temperatures.

Comparison of the data from two replicate studies presented in Tables 1 through 5 and the statistical analyses (Table 6) revealed that the presence of both oxygen and CO_2 was beneficial to food safety and detrimental to the pathogen. Most striking was the greater lethality at day 0 of ionizing radiation for *L. monocytogenes* in the presence of oxygen (compare results at day 0 in Tables 1 through 5 with values for the kGy terms in Table 6), but also important was the inhibition of the ability to multiply and possibly the ability to recovery from radiation injury of *Listeria* following CO_2, as is indicated by the lower value for the day term for 64% CO_2 compared with those values for 17.2 and 40.5% CO_2 atmospheres (Table 6). This effect is not just due to anaerobiosis, since multiplication of nonirradiated *Listeria* on turkey was equivalent under both air-permeable and vacuum packaging (compare Tables 1 and 2). Nor was there reduced survival of *L. monocytogenes* in 64.0 versus 17.2% CO_2 at time 0. (Compare the results for 2 kGy in Tables 3 and 5.)

Sante et al. *(21)* investigated the effects of several modified atmospheres (100% O_2, 100% N_2, 100% CO_2 + O_2 scavenger, and 25% CO_2:9% N_2:66% O_2) and of vacuum on the color stability and microbiology of nonirradiated turkey breast meat. The meat packed under either vacuum or 100% CO_2 + O_2 scavenger had the greatest color stability and lowest rate of myoglobin oxidation. The microbiological contamination of the meat stored under the CO_2 + O_2 scavenger was also lowest. We used sterile ground turkey meat that was inoculated with *L. monocytogenes* and found no significant differences between the multiplication of this pathogen in air-permeable or vacuum packaging. However, the differences between our results and those of Sante et al. *(21)* are possibly due to the mixed flora in their study and our use of *L. monocytogenes* in the absence of other microflora. Multiplication and/or recovery of irradiated *L. monocytogenes* was inhibited by 40.5 and 64% CO_2 MAP levels at a mild abuse temperature of 7°C when the results are compared with those obtained with either air-permeable or vacuum packaging. In nonirradiated samples, the inhibition of multiplication of *Listeria* by CO_2 was evident; this type of inhibition has been noted by others, though not during studies on sterile meat *(8, 9, 12, 17)*. This indicates that the inhibition applies to *L. monocytogenes* and is not associated with any other microflora that

TABLE 5. *Multiplication of* L. monocytogenes *on ground turkey at 7°C that was packaged and gamma-irradiated in an atmosphere of 64.0% CO_2 and 36.0% N_2 (means of two independent replicate studies, log CFU/g)*

	Day (mean ± SD)								
kGy	0	1.5	5.5	8.5	12.5	14.5	20.5	22.5	27.5
0	3.68 ± 0.09	3.72 ± 0.36	4.03 ± 0.29	4.88 ± 0.21	5.45 ± 0.31	6.47 ± 0.92	7.03 ± 1.50	8.18 ± 0.09	8.50 ± 0.10
0.5	3.01 ± 0.15	3.02 ± 0.20	2.96 ± 0.37	3.36 ± 0.60	4.70 ± 1.56	4.55 ± 0.33	6.50 ± 2.25	6.88 ± 0.79	8.08 ± 0.08
1.0	2.56 ± 0.28	2.55 ± 0.16	2.46 ± 0.09	2.62 ± 0.37	3.74 ± 0.25	3.17 ± 1.06	3.01 ± 0.75	5.54 ± 0.98	7.25 ± 1.65
1.5	2.02 ± 0.34	1.38 ± 0.53	1.86 ± 1.04	2.16 ± 0.90	2.17 ± 0.27	3.07 ± 0.75	2.55 ± 0.23	2.16 ± 0.00	5.36 ± 0.76
2.0	0.52 ± 0.00	0.76 ± 0.34	0.00 ± 0.00	0.26 ± 0.37	0.76 ± 0.34	0.00 ± 0.00	1.44 ± 1.30	0.52 ± 0.00	0.00 ± 0.00
2.5	0.00 ± 0.00	0.00 ± 0.00	0.41 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	1.92 ± 0.31	0.00 ± 0.00	0.52 ± 0.00	1.14 ± 1.60

TABLE 6. *Analyses of variances for inoculated turkey stored at 7°C for 27.5 days under different packaging atmospheres*

Atmosphere	Intercept	kGy	Day	kGy × Day	kGy^2	Day^2	R^2
Oxygen-permeable poultry pouch	3.963	−4.788	0.367	−0.109	1.235	−0.004	0.916
17.2% CO_2:82.8% N_2	3.583	−0.642	0.285	−0.040	−0.489	−0.002	0.909
40.5% CO_2:59.5% N_2	3.354	−0.444	0.235	−0.070	−0.476	0.000	0.922
64.0% CO_2:36.0% N_2	3.541	−1.421	0.125	−0.081	0.049	0.003	0.882
Vacuum	3.294	−1.019	0.377	−0.064	−0.265	−0.004	0.821

may be present. Wimpfheimer et al. *(25)* did not find *L. monocytogenes* multiplication at 10°C in raw chicken to be inhibited under MAP containing 72.5% CO_2 containing 5% oxygen but did find that its growth was inhibited by an anaerobic atmosphere containing 75% CO_2. Thus, under anaerobic conditions, their results were similar to ours, but they were not similar when the atmosphere contained 5% O_2. In our first study, we evaluated a similar atmosphere for its possible interaction with the inactivation of *L. monocytogenes* by ionizing radiation, but we did not evaluate the ability of these mixtures to inhibit the multiplication of the pathogen.

Grant and Patterson *(11)* observed that *L. monocytogenes* counts in irradiated (1.75 kGy), MAP (25% CO_2: 75% N_2) pork incubated at 10°C were significantly ($P < 0.01$) lower than in nonirradiated samples for 9 days. Our results extend the incubation time to 28 days and note a significant interaction between radiation dose and the concentration-dependent inhibition of *L. monocytogenes* multiplication by CO_2.

The results of Wimpfheimer et al. *(25)* suggest that the addition of oxygen to the MAP mixture might be counterproductive because of the inability of CO_2 to inhibit multiplication of *L. monocytogenes* in the presence of O_2. However, in contrast to the results obtained by Wimpfheimer et al. *(25),* Mano et al. *(18),* observed the extension of the lag phase of *L. monocytogenes.* It is not clear from either our results or those of Wimpfheimer et al. *(25)* whether CO_2 in the presence of oxygen will inhibit the recovery of *L. monocytogenes* from injury induced by gamma radiation. Our results indicate that *L. monocytogenes* is much more sensitive to gamma radiation in the presence of oxygen. Though CO_2 had little effect on the lethality of *Listeria* to gamma radiation, there was a concentration-dependent inhibition of the multiplication and/or of the recovery of irradiated cells. The results suggest that any *Listeria* that survived a dose of 2.5 kGy could be prevented from multiplying at mild abuse temperatures with the combined use of MAP with concentrations of CO_2 of 50% or higher. This conclusion must be tested by further experimentation in order to establish whether there is a quantitative relationship between the inactivation of *L. monocytogenes* by gamma radiation and O_2 concentration and in order to evaluate the ability of CO_2, in the presence of O_2, to inhibit the recovery of *L. monocytogenes* from radiation injury.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of K. Snipes and the editorial advice of C. Custer, J. Smith, and S. Thayer.

REFERENCES

1. Aldridge, C., P. W. Jones, S. F. Gibson, J. W. Lanham, M. C. Meyer, R. Vannest, and R. A. Charles. 1977. Automated microbiological detection/identification system. J. Clin. Microbiol. 6:406–413.
2. American Society for Testing and Materials (ASTM). 1996. E1607-94 Standard practice for use of the alanine–EPR dosimetry system, 12.02:838–843. *In* 1996 Annual book of ASTM standards. ASTM, West Conshohocken, Pa.
3. Avery, S. M., A. R. Rogers, and R. G. Bell. 1995. Continued inhibitory effect of carbon dioxide packaging on *Listeria monocytogenes* and other microorganisms on normal pH beef during abusive retail display. Int. J. Food Sci. Technol. 30:725–735.
4. Draper, N. R., and H. Smith. 1981. Applied regression analysis, 2nd ed. John Wiley and Sons, Inc., New York.
5. Elliott, P. H., R. I. Tomlins, and R. J. H. Gray. 1985. Control of microbial spoilage on fresh poultry using a combination potassium sorbate/carbon dioxide packaging system. J. Food Sci. 50:1360–1363.
6. Epp, E. R., H. Weiss, and A. Santomasso. 1968. The oxygen effect in bacterial cells irradiated with high-intensity pulsed electrons. Radiat. Res. 84:320–325.
7. Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging technology—a review. J. Food Prot. 54:58–70.
8. Farber, J. M., Y. Cai, and W. H. Ross. 1996. Predictive modeling of the growth of *Listeria monocytogenes* in CO_2 environments. Food Microbiol. 32:133–144.
9. Farber, J. M., and E. Daley. 1994. Fate of *Listeria monocytogenes* on modified-atmosphere packaged turkey roll slices. J. Food Prot. 57:1098–1100.
10. Freund, R. J., R. C. Little, and P. C. Spector. 1986. SAS system for linear models. SAS Institute, Inc., Cary, N.C.
11. Grant, I. R., and M. F. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbiological safety of minced pork stored under temperature abuse conditions. Int. J. Food Sci. Technol. 26:521–533.
12. Hart, C. D., G. C. Mead, and A. P. Norris. 1991. Effects of gaseous environment and temperature on the storage behaviour of *Listeria monocytogenes* on chicken breast meat. J. Appl. Bacteriol. 70:40–46.
13. Hastings, J. W., W. H. Holzapfel, and J. G. Niemand. 1986. Radiation resistance of lactobacilli isolated from radurized meat relative to growth and environment. Appl. Environ. Microbiol. 52:898–901.
14. Hollaender, H., G. E. Stapleton, and F. L. Martin. 1951. X-ray sensitivity of *E. coli* as modified by oxygen tension. Nature 167:103–104.
15. Hotchkiss, J. H., R. C. Barker, and R. A. Qureshi. 1985. Elevated carbon dioxide atmospheres for packaging poultry. II. Effects of chicken quarters and bulk packages. Poult. Sci. 64:333–340.
16. John, P. W. M. 1971. Statistical design and analysis of experiments. Macmillan, New York.
17. Lee, M., J. G. Sebranek, D. G. Olson, and J. S. Dickson. 1996. Irradiation and packaging of fresh meat and poultry. J. Food Prot. 59:62–72.
18. Mano, S. B., G. E. Garcia De Fernando, D. Lopez-Galvez, M. D. Selgas, M. L. Garcia, M. I. Cambero, and J. A. Ordoñez. 1995. Growth/survival of natural flora and *Listeria monocytogenes* on re-

frigerated uncooked pork and turkey packaged under modified atmospheres. J. Food Saf. 15:305–319.
19. Marshall, D. L., L. S. Andrews, J. H. Wells, and A. J. Farr. 1992. Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* on precooked chicken. Food Microbiol. 9:303–309.
20. Patterson, M. 1988. Sensitivity of bacteria to irradiation on poultry meat under various atmospheres. Lett. Appl. Microbiol. 7:55–58.
21. Sante, V., M. Renerre, and A. Lacourt. 1994. Effect of modified atmosphere packaging on color stability and on microbiology of turkey breast meat. J. Food Qual. 17:177–195.
22. SAS Institute, Inc. 1987. SAS/STAT© guide for personal computers, version 6. SAS Institute, Inc., Cary, N.C.
23. Soffer, T., P. Margalith, and C. H. Mannheim. 1994. Shelf-life of chicken liver egg pate in modified atmosphere packages. Int. J. Food Sci. Technol. 29:161–166.
24. Thayer, D. W., and G. Boyd. 1991. Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile, mechanically deboned chicken meat. Poult. Sci. 70:381–388.
25. Wimpfheimer, L., N. S. Altman, and J. H. Hotchkiss. 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11:205–214.
26. Zelle, M. R., and A. Hollaender. 1955. Effects of radiation on bacteria, p. *In* A. Hollaender (ed.), Radiation biology, vol. II. Ultraviolet and related radiations. McGraw-Hill Book Company, Inc., New York.